Tumour markers

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Laboratory examination in patients with tumours

- **Blood count**
- **Basic biochemical parameters** – various changes (inflammatory markers, nutrition, metastases – liver, bones – calcium, expansion of tumour - ureter, tumour degradation – uric acid etc.)
- **Tumour markers** – no universal marker
- …

Tumour markers

- **Substance present in the tumour, produced by the tumour or by the organism as a response to the presence of the tumour**
- Provide information about biological characteristics of the tumour
- **Qualitative determination** – histopathologic, in the tunour tissue
- **Quantitative determination** – in the serum or biological fluids, dynamic follow-up

Tumour markers - history

- 30-ies of the 20th century – **hCG** (physiologically produced by placenta) discovered in young men with testicular tumours *(Zondek)*
- 70-ies of the 20th century - **α_1-fetoprotein** discovered in liver tumours in mice *(Tatarinov)*, later on described in human hepatomas *(Abelev)*
- Further intensive research and their practical **usage of markers in oncology and prenatal diagnostics**

- **EGTM – European Group on Tumour Markers**

Tumour markers

- **Soluble markers** – classical tumour markers , various chemical substances
- **Circulating cellular elements** – circulating tumour cells, circulating endothelial cells and their precursors
- **Genetic abnormalities** – detection of mutations in oncogenes and tumour supressor genes, protein products of oncogenes, further changes

Chemical characteristics of TU markers

- **Enzymes** – PSA, NSE,TK, LDH
- **Immunoglobulins** – IgG, IgM, IgA, β_2-microglobulin, free light chains
- **Hormones** – growth hormon, ACTH, TG, PRL, calcitonin, PTH, hCG
- **Cytokeratines** (soluble derivatives) – tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS), fragment of cytokeratine 19 (CYFRA 21-1)
- **Glycoproteins, glycolipids and saccharides** – AFP, hCG, CEA, squamous cell carcinoma antigen (SCC), CA 19-9, CA 125, CA 15-3, CA 549, CA 72-4
- **Receptors** – estrogen and progesteron receptors, HER2/neu, EGF

Tumour markers – clinical-chemical division

- Oncofetal antigens
- Tissue and organ specific antigens
- Non-specific antigens

Oncofetal antigens

- Substances produced during the fetal period or by placenta, postnatally low concentration and increase in connection with some disease, mainly tumours.

  Antigens that appear soon in the ontogenesis and postnatally characteristic for less differentiated (i.e. more malignant) tumours.

  α_1-fetoprotein (**AFP**)

  human chorionic gonadotrophin (**hCG**)

  carcinoembryonic antigen (**CEA**)

  placental alkaline phosphatase (**PLAP**)

Tissue and organ specific antigens

- **Physiologically** present in healthy tissue or organ, outside released only in minimal amounts
- **Pathological states** (tumours, inflammation, injury) – increased release

  prostatic specific antigen (**PSA**), neuron specific enolase (**NSE**), protein **S-100**, soluble fragments of cytokeratins (**TPA, TPS, CYFRA 21-1**), **CA** antigen defined by monoclonal antibodies, squamous cells carcinoma antigen (**SCC**), thyreoglobulin (**TG**), hormones and their precursors in tumours from glands which produce them physiologically (e.g. C-peptid in insulinoma)

Non-specific antigens

- enzymes and hormones produced by tumours (tumours from organs which do not produce them physiologically – **paraneoplastic production**), reaction to the presence of tumour

  ferritin, lactate dehydrogenase (**LDH**), thymidinkinase (**TK**), β_2-microglobulin,

  some acute phase reactants,

  lipid associated sialic acid (**LASA**)

  lung tumours – ACTH, ADH, parathormon etc.

- **AFP (α_1-fetoprotein)** – glycoprotein structurally similar to albumin, physiologically produced by yolk sack, later by fetal liver. Used for dg and monitoring of hepatocellular carcinoma and germ cells testicular and ovarian, also in prenatal screening of Down syndrome in the 2nd trimester of pregnancy.
- **CEA** – glycoproteins with high saccharides content, MW 180 kDa, present in fetal intestine, used for monitoring of colorectal CA, event. other CA (breast, lung), higher levels in smokers.
- **Human chorionic gonadotrophin (hCG)** – glycoprotein, α and β subunits non-covalently bound, α subunit identical with LH, FSH and TSH.

  Indication of examination: dg of pregnancy (hCG), prenatal screening of Down syndrome (free β hCG), monitoring and prognosis of germ cell tumours, trophoblastic disease (β hCG – specific hCG)

- **CA 125** – monitoring of ovarian CA
- **CA 15-3** – monitoring of breast CA
- **CA 72-4** – monitoring of gastric CA
- **CA 19-9** – glycolipid, determinant of blood group Lewis a (5% of population does not produce it), for monitoring of pancreas CA (and bile ducts), CAVE – contamination by saliva
- **CYFRA 21-1** – soluble fragment of cytokeratine 19, for lung CA (non-small cell) and urinary bladder
- **NSE** – for monitoring of small cell lung cancer, neuroblastoma, apudoma, CAVE – hemolysis
- **PSA** – serin protease, glycoprotein, monitoring of prostata CA, CAVE – preanalytical phase

  ratio fPSA/PSA, velocity, density

- **SCC** – squamous cell carcinoma antigen, monitoring of head and neck tumours, genital tumours and oesophagus tumour
- **TPA** – tissue polypeptide antigen, mixture of soluble cytokeratines 8, 18 and 19, monitoring of CA of urinary bladder
- **TPS** – tissue polypeptide specific antigen, soluble fragment of cytokeratine 18, monitoring of metastasing breast CA
- **TK** – thymidinkinase, marker of proliferation, leukemias
- **β_2-microglobulin** – hematological malignancies (NHL), influenced by renal function
- **Ferritin** – hematological malignancies
- **Paraprotein, free light chains** – monoclonal gamapathy (urine – Bence-Jones protein, not determined by the dip stick test)

- **S100B** – malignant melanoma
- **Chromogranin A** – neuroendocrine tumours
- **Isoenzyme of pyruvate kinase** – kidney cancer

- **Estrogen receptors** – prediction of the effect of hormonal therapy in breast cancer, **determination in the tumour tissue**
- **Progesteron receptor** – prediction of the effect of hormonal therapy in breast cancer, **determination in the tumour tissue**

Tumour markers – recommened use according to localization and tumour type

- **Stomach** - CA 72-4, CEA
- **Oesophagus**
  - Cranial part - SCCA (CYFRA 21-1)
  - Lower part - CA 72-4, CEA
- **Pancreas** - CA 19-9, CEA
- **Liver**
  - AFP, CEA
  - cholangiocellular - CA 19-9
  - metastases - CEA

Tumour markers – recommened use according to localization and tumour type

- **Breast** - CA 15-3, CEA (TPA/S)
- **Lung**
  - SCLC - CEA, NSE (TPA/S)
  - NSCLC - CYFRA 21-1, CEA (SCC)

Tumour markers – recommened use according to localization and tumour type

- **Ovary**
  - non-mucinous - CA 125 (TPA/S)
  - mucinous - CA 19-9, CA 72-4 (CEA)
  - germinative - AFP, hCG
- **Cervix**
  - epidermoid - SCCA (CYFRA 21-1, CEA)
  - adenocarcinomas - CEA
- **Corpus uteri** - CA 125 (CEA)
- **Vulva** - SCCA

Tumour markers – recommened use according to localization and tumour type

- **Kidney** - TPA/S, CEA (NSE)
- **Urinary bladder** - TPA/S (CYFRA 21-1)
- **Prostate** - PSA, fPSA (ChgA)
- **Testes**
  - seminomas - hCG, AFP (NSE)
  - non-seminomas - hCG, AFP

Tumour markers – recommened use according to localization and tumour type

- **Karcinoid** - 5-hydroxy, 3-indolylacetic acid, NSE
- **Thyroid gland**
  - medullar CT, CEA (NSE)
  - anaplastic TPA/S
- **Melanoma** - NSE, S100beta (TK)
- **Head, neck** - SCCA (CYFRA 21-1)
- **CNS**
  - neuroblastomas - NSE
  - gliomas - CEA
  - astrocytomas - TK

Tumour markers – recommened use according to localization and tumour type

- **Leukemia** - TK, FER, LD
- **Lymfoma**
  - Hodgkin - B2M, FER, LD
  - non-Hodgkin - TK, B2M, LD
- **Multiple myeloma** - B2M, paraproteins

Determination of tumour markers

- Indication
- Preanalytical phase
- Determination – metods, interferences
- Interpretation

Determination of tumour markers

- **Immunochemistry**
  - radio immune assay – RIA, IRMA
  - enzyme immune assay - ELISA, EIA, MEIA
  - fluorescence assay - FPIA, TRACE
  - chemiluminiscence assay - CLIA
- Use the same diagnostic kit from the same company!!! *(or rebaselining)*

Determination of tumour markers - analytical interferences

- Cross reactivity of structurally similar molecules
- Hook-effect caused by high concentration of the marker
- Carry-over of analyzed marker between samples
- Interference of heterophil and human anti-mouse antibodies (HAMA)
  - Check the same samples by different analytical technology.

Indication and interpretation of tumour markers

- **Not for diagnostics but for monitoring.**

  They can help in the diagnostic process.
- Positive finding of tumour markers is of diagnostic value, negative finding does not exclude a tumour!!!

  For diagnosis, histopathological examination and additional TU markers determination is decisive.

  Transient elevation of a tumour marker – inflammation, non-malignant tumour, trauma, after efficient therapy, in decreased renal or liver function for markers which are eliminated this way
- **Screening** – faecal blood test, discussed PSA – not yet

  u specific populations – calcitonin in familes with medullar CA of the thyroid gland, CA 15-3 in BRCA mutations

Indication and interpretation of tumour markers

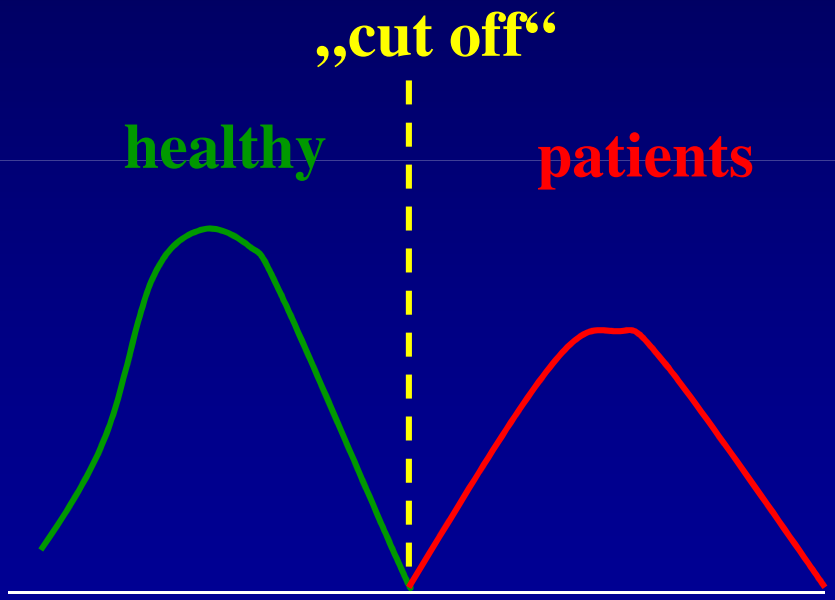
- **Dynamics of changes** (increase, although in reference range may indicate a recidive sooner than visualization by CT, US, PET)

  increase in 3 consecutive blood collections or increase by more than 25% is significant

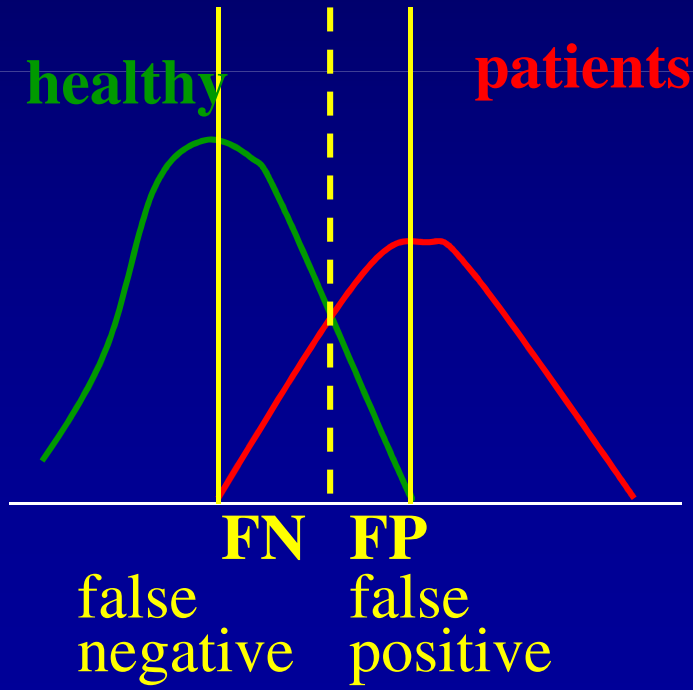
  TU marker may detect a tumour of 1 mg (10^6 malignant cells), clinical diagnosis is possible for 10^9 malignant cells
- **Systematic examination** – repeated determination after operation, at the beginning shorter intervals, later cca 3-6 months)
- **Follow up of more tumour markers** – higher probability of detection of a tumour

Evaluation of TU markers

- Ideal situation



- Reality



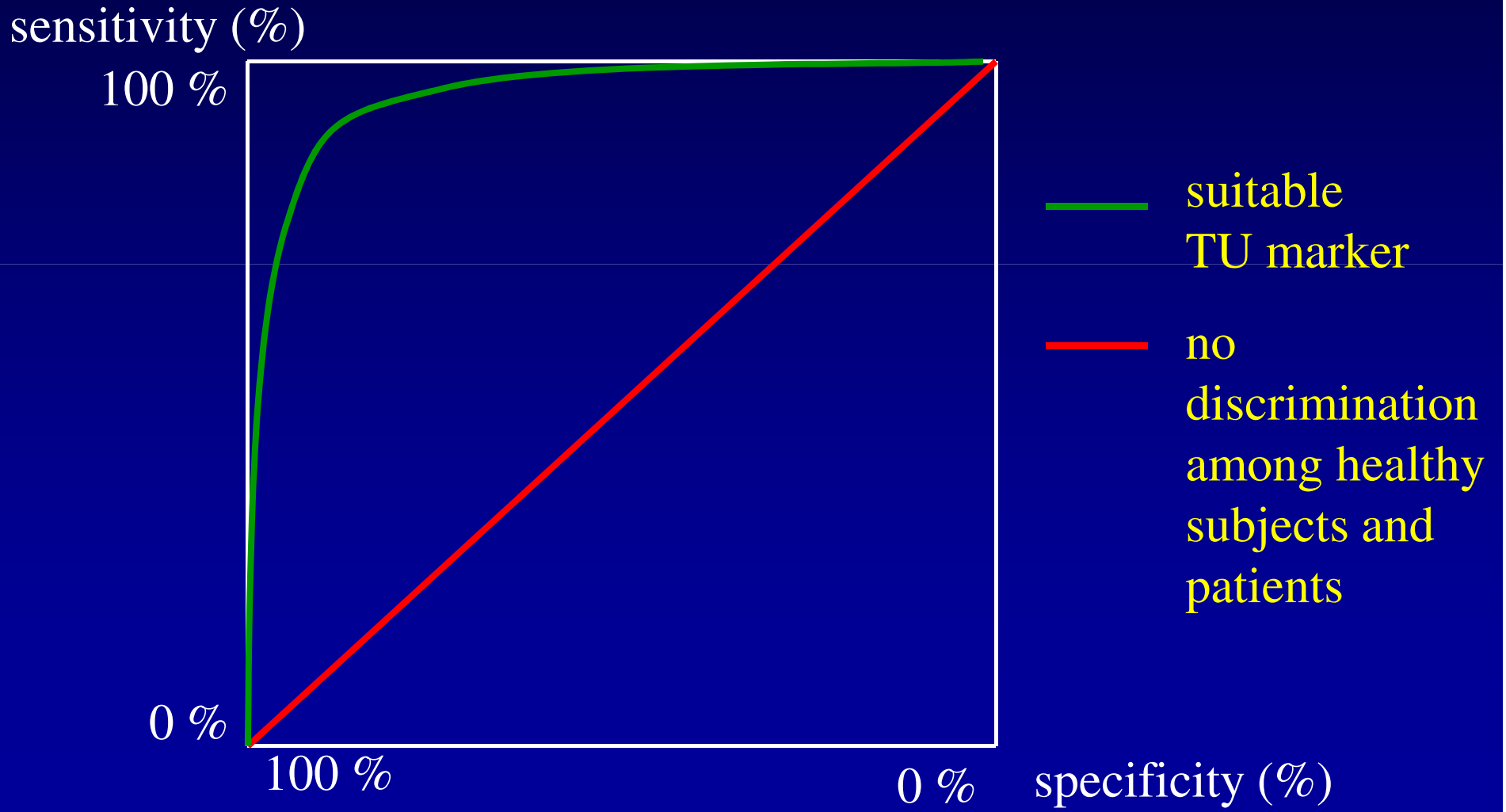
Evaluation of TU markers

- **Specificity** = TN / (TN + FP)
  probability that a negative test means negative dg
  true negativity in healthy subjects
- **Sensitivity** = TP / (TP + FN)
  probability that a positive test means a positive dg
  true positivity in patients with tumours
- **Positive predictive value** = TP / (TP + FP)
- **Negative predictive value** = TN / (TN + FN)

TP – number of true positive examinations
TN – number of true negative examinations
FP – number of false positive examinations
FN – number of false negative examinations

Evaluation of TU markers using ROC curves

(ROC = receiver operating characteristic)



Ideal marker

- **High specificity** – not present in other diseases - non-tumours and in healthy subjects
- **High sensitivity** – detectable at the beginning of the disease
- Optimal positive and negative predictive value
- Organ specific
- Correlation with the tumour mass and prognosis

does not exist…so far

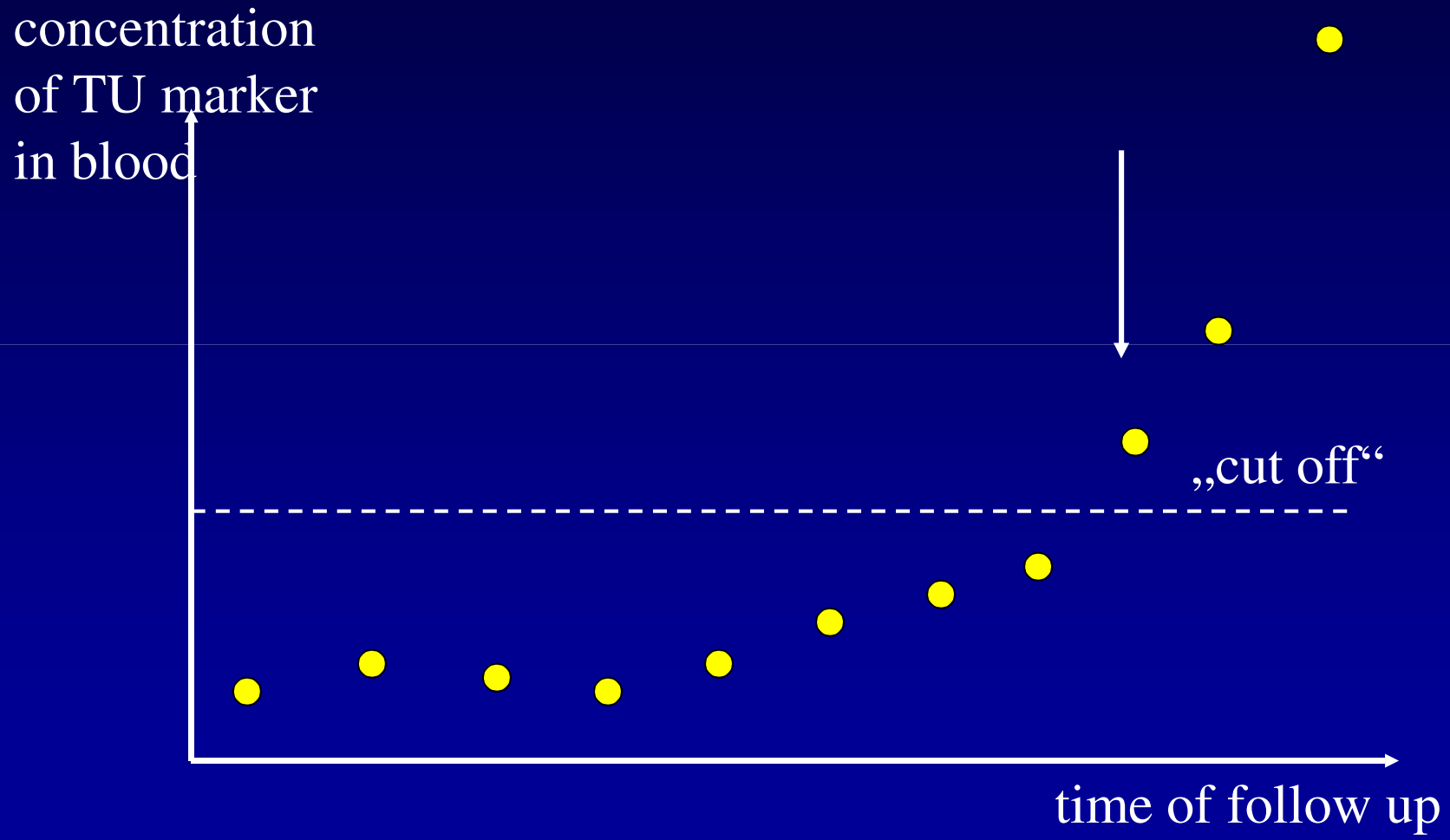
Example
CEA for colorectal CA

- 95% specificity – i.e. 5% of healthy subjects are falsly regarded as patients with tumours
- 70% sensitivity – i.e. does not detect 30% of patients with tumours

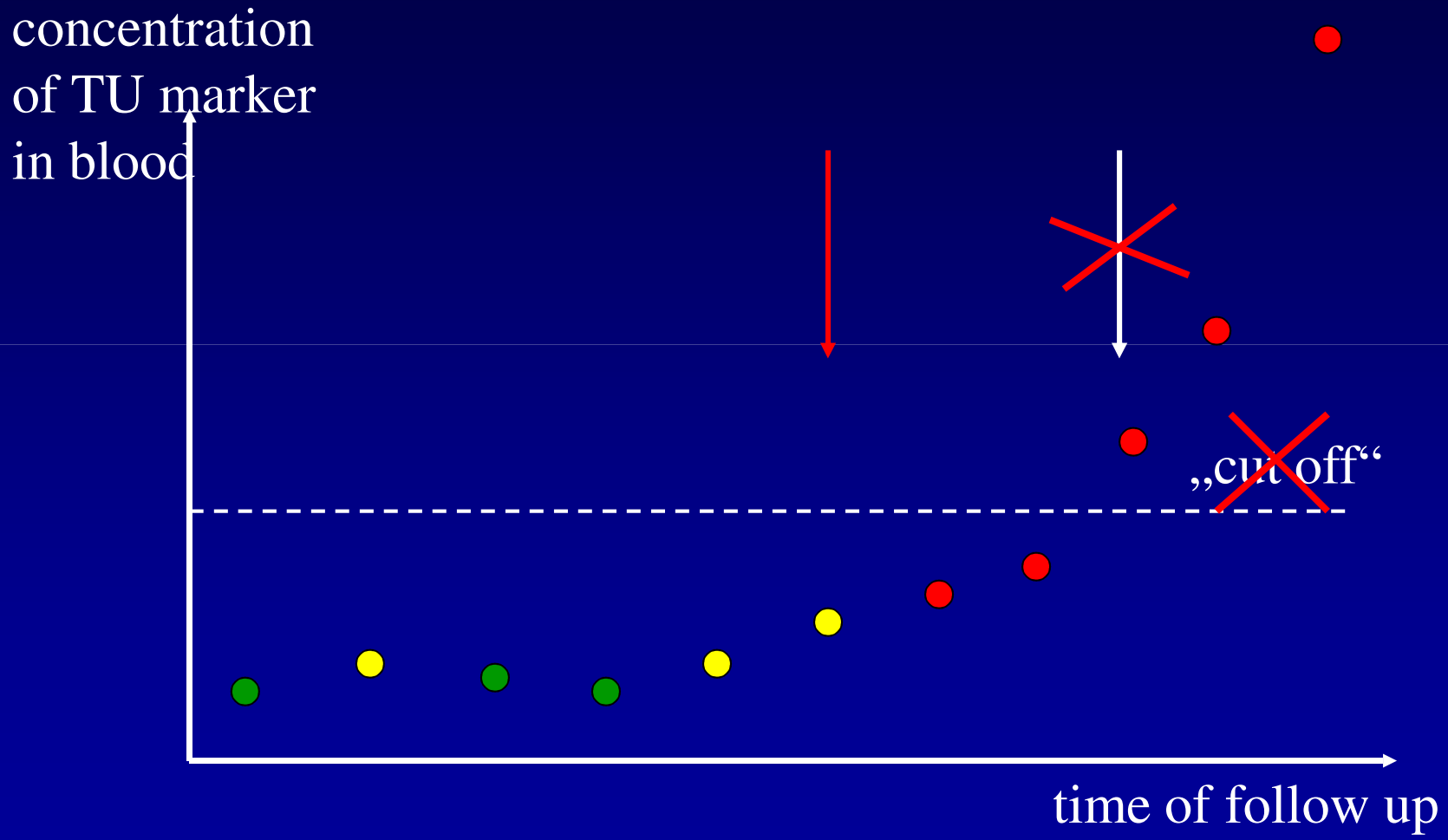
Interpretation of results of TU markers

- In the past – comparison with reference range (might be suitable for a unique determination of unknown patient)
- Today recommended **determination of individual baseline values** (concentration of a TU marker in „stabilized“ status, i.e. after operation – extraction of the tumour mass) and systematic **dynamic follow up**

Dynamic follow up



Dynamic follow up



Molecular biology in diagnostics of tumours

- Tumours – mutations of genes which products regulate cell proliferation, development, differentiation and cell death
- Oncogenes and antioncogenes

Oncogenes and their significance in tumour - examples

- **abl** → tyrosin-protein kinase (leukemias)
- **erb B1, B2** → receptors for epidermal growth factor
- **c-myc** – transcription factor (lymphomas)
- **neu(erbB-2)** → receptor for epidermal growth factor (breast CA)
- **NF1** – nuclear factor
- **ras** → GTP-ase activating protein

Antioncogenes and their significance in tumour - examples

- **BRCA 1 and BRCA 2** – reparation of DNA defects (breast and ovarian CA)
- **p53** – regulation of the cell cycle
- **RB1 a RB2** – regulation of the cell cycle (retinoblastoma)

Potential new tumour markers

Proteins and oncoproteins – products of mutated genes which play a role in cell life, their division, differentiation and metastasing

- **Regulation of the cell cycle** - cyclins
- **Apoptosis** – Bcl-2 protein, sFas, protein-product of mutated gene p53
- **Signal transduction** - c-erbB-2 (Her-2/neu), EGRF, IGF, TNF-α
- **Adhesion** - ICAM-1, VCAM-1
- **Angiogenesis** – inhibitors of angiogenesis - angiostatin, angiogenin, trombospondin
- **Markers associated with specific characteristics of tumour cells** – matrix metalloproteinases, urokinase plasminogen activator (uPA) and its inhibitor (PAI-1)

New and potential tumour markers

- free DNA in plasma (and microsatelite changes)
- free mRNA in plasma
- enzymes of DNA synthesis in tissue samples
- mammaglobin - breast cancer
- heparanase
- …

Literature

- Guidelines of the Czech Society of Clinical Chemistry – www.cskb.cz
- Guidelines of the European Group for Tumour Markers (EGTM) – www.egtm.eu